SCIENCE

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MSS. intended for publication and books, etc., intended for review should be sent to the responsible editor, Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

AMERICAN SOCIETY OF BACTERIOLOGISTS.

THE third annual meeting of the Society was held at Chicago University on December 31, 1901, and January 1, 1902. The President of the Society was Professor W. H. Welch, of Johns Hopkins University. The following are abstracts of the papers presented at the three sessions of the Society:

Conditions affecting the Thermal Death-point of Bacteria in Milk: H. L. RUSSELL and E. G. HASTINGS, State University, Madison, Wis.

The authors have tested the resistance of bacteria in the surface pellicle ('scalded layer') that forms on milk when it is heated to temperatures of 60°C. and above. They confirmed under commercial conditions the fact demonstrated by Theobald Smith under laboratory conditions that the resistance of the tubercle organism is materially increased when milk is heated in contact with the air. In order to demonstrate this increased resistance more clearly they further experimented with a peculiarly resistant coccus that they had found in milk, which had, in a vegetative stage, a thermal death-point of 75°C. when exposed for ten minutes in sealed tubes. In open tubes the organism retained its vitality as high as 82°C. When surface membranes were removed and plated on agar, colonies developed from them, but not from samples

of the milk below. The increased resistance is not due to lowered temperature at surface, as was shown by removing membrane and placing same in water, when it sunk to the bottom. The protection therefore afforded the bacteria is due to the nature of the membrane itself, preventing the heat from exercising the usual effect.

The Comparative Growth of Bacteria in Milk: H. W. Conn, Wesleyan University, Middletown, Ct.

This paper described a series of experiments, the design of which was to determine what species of bacteria develop in milk during the first twenty-four hours and what species disappear. The general purpose of the experiments was to determine as far as possible the relation of milk bacteria to the healthfulness of milk. The conclusions presented by the paper were as follows: (1) Milk freshly drawn from the cow contains a large variety of bacteria. (2) For the first six hours and sometimes more, there is no increase in the number of bacteria, even when the milk is kept at 70°. On the contrary, there is commonly a decrease due to what has been called the 'germicide power' of milk. (3) In the fresh milk the largest number of bacteria are streptococci, which come, in most cases, directly from the udder of the cow. (4) During the first forty-eight hours there is a very great increase in the number of bacteria, but the number present after one or two days' growth is quite independent of the number present at the start. In many cases milk, which when fresh contained a small number of bacteria, at the end of forty-eight hours contained a number far greater than other samples of milk which at the outset had a larger number of bacteria present. (5) During the first forty-eight hours there is a considerable increase in the number of streptococci, followed by their decrease and final disappearance. (6) At the outset the number of lactic bacteria is extremely small, so small as, at times, quite to escape observation. (7) These lactic bacteria are, at least in the series of experiments described, derived from sources external to the cow and never, or rarely, from the milk ducts. (8) The lactic bacteria, though very few in number at the outset, increase far more rapidly than any other types, so that within twenty-four hours they are commonly in the majority, and by the end of forty-eight hours they commonly comprise considerably over ninety per cent. of all the bacteria present.

Rusty Spot in Cheddar Cheese: H. A. Harding and L. A. Rogers, N. Y. Agricultural Experiment Station, Geneva, N. Y.

Rusty spot is a bacterial trouble of cheddar cheese characterized by reddish-yellow discolorations scattered in points or blotches throughout the mass. The cheese does not become poisonous and the flavor is not affected, but the market price is reduced on account of the unusual appearance. This trouble is confined to a few factories, but a considerable part of their output is affected. A short, plump, causal bacillus was isolated by Cornell in 1898 and called *Bacillus rudensis*. By the addition of pure cultures to vats of milk he was able to reproduce the discoloration in the resulting cheese. In practice, the main source of trouble is the bacterial growth upon the factory utensils. However, there are often outside foci in connection with the dairies, capable of reseeding the factory after it has been once freed from the infection by a careful cleaning and disinfection. The direct application of steam to all the factory utensils on three days each week was tried in three infected factories during the past season. This is most easily accomplished by placing all

the small utensils in the large vat, drawing a canvas cover over the same and forcing steam into the vat for twenty minutes. This treatment was highly successful in two factories and fairly so in a third where the outside influences were quite unfavorable.

On the Apparent Identity of the Cultural Reactions of B. coli communis and Certain Lactic Bacteria: S. C. PRESCOTT, Massachusetts Institute of Technology, Boston, Mass.

While engaged in an examination of certain lactic bacteria, the author was impressed with the similarity presented by some of the cultures to *B. coli communis,* and has carried on investigations with a large number of lactic-acid-producing organisms, comparing their cultural reactions to those of *B. coli.* He defines the colon bacillus as a short, motile rod of intestinal origin, which forms thin, irregular films upon the surface of gelatin; produces no liquefaction; gives nail growth in stick cultures; a whitish translucent layer upon agar; a more or less abundant, moist, yellowish growth upon potatoes; produces turbidity and some sediment in broth; ferments dextrose and lactose with the formation of gas; reduces nitrates to nitrites; coagulates milk; reduces litmus with subsequent slow return of the color, and produces indol.

The lactic acid group is broadly defined as consisting of those bacteria in which the ability to bring about the fermentation of sugars to lactic acid is strongly developed. Forty-seven cultures were isolated from the following sources: bran (7), fresh meat (3), sour milk (4), flour (2), cornmeal (6), buckwheat (7), barley (4), butter culture (3), an acid-producing organism in technical use (5), and a breakfast food (2). All these were tested in the following particulars: growth on litmus, lactose agar, gelatin agar, milk, dextrose broth, nitrate solution, Dunham's solution, bouillon and potato; and were further compared morphologically with relation to motility and spore formation, and with relation to air. Of the forty-seven cultures examined, twenty-five gave the typical colon reactions; six gave the tests weakly or failed in one test only, while the others failed in a greater degree. Some of the bacteria from most of the sources gave typical colon reactions.

The author points out two views that may be entertained regarding these bacilli: (1) They may be true colon bacilli from sources which can only be conjectured, or (2) they may be lactic acid organisms, not absolutely identical with the colon bacilli, but yet almost impossible to differentiate from them. The latter view the author regarded as more probable. Of great importance is the fact that had they been isolated from water they would have been undoubtedly regarded as colon bacilli. Hence the work has a very practical sanitary bearing and indicates that too much reliance must not be placed upon the so-called colon test of potable waters.

Oysters and Sewage in Narragansett Bay: CALEB A. FULLER, Brown University, Providence, R. I.

The city of Providence discharges, daily, about 14,000,000 gallons of sewage into upper Narragansett Bay, chiefly through a single main. This sewage is carried down the bay by tide and comes into more or less direct contact with some of the oyster beds. Samples of water and oysters were collected from different localities in the bay, and analyses made before the material was six hours old. The ordinary tests for sewage contaminations were used, the fermentation tube, carbol broth and litmus lactose agar.

The results showed: (1) That water,

oysters, mussels and clams from a point one quarter of a mile distant from the sewer opening contained *B. coli, B. cloacæ* and *Bact. lactis aërogenes.* (2) That water and oysters from a bed two miles below the sewer contained the same organisms. (3) That thirty per cent. of the oysters and about sixty per cent. of the water samples from a bed situated in a strong tidal current, about five miles from the sewer, contained *B. coli.* (4) That forty per cent. of the oysters and seventy per cent. of the water samples from a bed in sluggish water, five and a quarter miles from the sewer, contained *B. coli.* (5) That oysters from a bed six miles below the sewer contained *B. coli.* (7) That oysters from a bed six miles and one half below the sewer contained no colon bacilli; the water contained *B. coli* only occasionally and then on a falling tide. (7) That beds still farther down the bay were entirely free from contamination.

Toxicity of Water toward Pathogenic Bacteria and the Possible Significance of the same in the Spontaneous Purification of Polluted Waters: H. L. Russell, Madison, Wis.

The preliminary data here reported have to do with the action of natural water on the vitality of various bacteria, particularly pathogenic organisms. When typhoid and colon organisms (several cultures of each) were inoculated in boiled waters (surface, deep well, spring) growth generally occurred. This was more marked with the colon than with the typhoid, and was more pronounced where the seeding was light. When the same cultures were exposed to the action of water filtered through a Chamberland or Berkefeld filter, or to etherized waters in which the anæsthetic had been removed by aspiration, growth not only did not take place, but the numerical content was greatly reduced, so that the cultures were often sterile in twenty-four hours. Further test showed that this toxicity of filtered water was lost when heated to about 60° C. for ten minutes. The origin of these toxic substances is ascribed to the development of water bacteria, as shown by taking boiled lake water and seeding the same with water bacteria. After incubation for thirty-three days, this water was again filtered and found toxic for typhoid and colon, which toxicity was again lost by reheating. Some bacterial species develop luxuriantly in standing water and, in some of the cases, it was found that these grew quite rapidly in filtered water, indicating their ability to tolerate the toxins. The relation of this toxicity to the destruction of pathogenic and fæcal organisms in water was suggested.

The Bacteria of the Ames Sewage-disposal Plant: L. H. Pammel, Agricultural College, Ames, Iowa.

The Ames sewage disposal plant has been in operation since 1898. During this time bacteriological, chemical and temperature records have been kept; account also has been taken of the flow of raw sewage and the effluent. These records indicate that this form of sewage disposal is a most efficient one, and is adapted for many inland towns. The average number of bacteria per c.c. in the effluent from January to December, 1899, inclusive, was 5,127. For the year 1900 the record is somewhat incomplete, arising from an unavoidable loss by fire; but from January, 1900, to September, 1900, inclusive, it was 5,414, having been as efficient as the previous season, the smallest number having been found during August, when there were 546. In January the average was 830; in September, 850. The average number of bacteria in the manhole, from August, 1899 to September, 1900, inclusive, was 639,720.

The average number of bacteria in tank during the same period was 446,611. The records during 1901 are as follows: Average number of bacteria per c.c. in effluent, 14,785; average number of bacteria per c.c. in manhole, 1,318,328; the average number of bacteria per c.c. in tank, 1,667,522.

No effort has been made to study a large number of different forms found in the effluent. *B. cloacæ, B. coli communis, B. liquefaciens fluorescens* and *Sarcina aurantica* have been found. *Bacillus prodigiosus* has also been found. This was introduced from the Manhattan sewage in 1900. That season it was found three times in the tank on June 19, in the east effluent on June 22, and in the west effluent on June 27. It was not found again till August, 1901, when it appeared in the effluent and continued to appear until the first of September. Various media were used. The Nüterstoff Heyden did not prove better than either pepton agar-agar or pepton gelatin. The blue litmus-agar and gelatin are excellent for differentiation.

On the Germicidal Action of the Organic Peroxides: Drs. F. G. NOVY and P. C. FREER, University of Michigan, Ann Arbor, Mich.

The investigation of the authors was begun with the object in view of finding the correct explanation of the action of metals and of sunlight upon bacteria. As is well known, certain metals, such as gold and copper, exert a marked inhibiting and even germicidal effect upon some bacteria. The studies of Miller, Behring and Bolton, Thiele and Wolf, have fully established the above-mentioned fact, but the interpretation of the results has not been wholly satisfactory. The fact that various surfaces, such as metals and fabrics, exert a marked effect upon the formation of benzoyl acetyl peroxide was established by the authors and served as a basis for the view that metals act upon bacteria by giving rise to energetic peroxides, which, of necessity, must be more active than ordinary peroxides. The action of sunlight has been ascribed by different workers to hydrogen peroxide, but the destructive action observed is greater than that which can be credited to this body. In order to substantiate the theory of the authors regarding the action of metals and of sunlight, it was deemed necessary to investigate the action of a number of known organic peroxides. The results show that some of these bodies, such as aceton peroxide and dibenzoyl peroxide, are wholly inert.

On the other hand, solutions of diacetyl, benzoyl acetyl, and of benzoyl hydrogen peroxides, and of phthalmonoperacid, exert pronounced and even remarkable germicidal properties. With reference to diacetyl peroxides and benzoyl acetyl peroxide, it was shown that the bodies themselves are chemically and bacterially inert, but on contact with water they undergo hydrolysis and give rise to the extremely energetic acetyl hydrogen and benzoyl hydrogen peroxides.

A solution of these peroxides (1: 3,000) is capable of destroying all pathogenic bacteria, and even such resisting spores as those of the potato bacillus, within one minute. Cholera and typhoid germs added to tap water are promptly destroyed by the addition of one part of peroxide to 100,000 parts of water. The authors point out the probable value of these peroxides in the prevention and cure of these and allied diseases. The destruction of bacteria in the mouth and saliva takes place with extraordinary rapidity and the reagents have shown themselves useful in diseases of the mouth.

The powerful effects of the organic peroxides is not explainable as due to nascent oxygen, since a solution of hydrogen peroxide, which will produce equal germicidal

action, contains one or even two hundred times as much nascent oxygen. The authors incline to the belief that the acetyl and benzoyl ions are the active agents.

Full papers upon this subject will appear in the *Journal of Experimental Medicine* and in the *American Journal of Chemistry.*

The Etiology of Yellow Fever: Walter Reed, M.D., Surgeon U. S. Army, and James Carroll, M.D., Contract Surgeon, U. S. Army.

In former contributions to this subject the authors have shown by observations made on human beings that yellow fever may be produced in the non-immune individual either by the bite of the mosquito (genus *Stegomyia*) or by the subcutaneous injection of a small quantity of blood (0.5 to 2 c.c.) drawn from the general circulation of a patient suffering with this disease. Thus far, however, microscopic examination of the blood, as well as of the bodies of infected mosquitoes, has proved negative. Cultures taken from the blood during the active stages of the disease also have yielded equally negative results. Leaving out of consideration, therefore, for the present the further microscopical search for the specific agent, both in the blood of the sick and in the bodies of infected mosquitoes, the authors presented some additional observations bearing on the etiology of the disease. In conducting these experiments they have been guided by the observations of Loeffler and Frosch on the foot and mouth disease of cattle, wherein it was conclusively demonstrated that the specific agent of this disease was so small as to readily pass through the pores of a porcelain filter.

Adopting the same line of procedure, it was ascertained that in yellow fever the blood serum which has been filtered through a Berkefeld laboratory filter is still capable of producing this disease when subcutaneously injected in small quantity (1.5 c.c.) into non-immune human beings. The authors reported an attack of yellow fever after the usual period of incubation in two out of three individuals thus treated, and further stated that the blood drawn from one of the cases produced by the injection of the filtered serum was capable of producing an attack in a third individual, when injected in small quantity; thus proving that the specific agent had really passed through the filter. They were also able to show that the blood in yellow fever, when heated to a temperature of 55°C. for ten minutes is quite innocuous if injected into susceptible individuals. The specific agent of yellow fever therefore is destroyed or markedly attenuated by this degree of heat.

Brain Abscess in Typhoid Fever due to Bacillus typhosus: R. W. McClintock, Rush Medical College, Chicago, Ill.

There are mentioned in the literature up to August, 1901, nineteen cases of meningitis and five of abscess of the brain in connection with typhoid fever, *Bacillus typhosus* having been present in all the cases of meningitis, but not found in any case of abscess of the brain.

Clinical History.—Temp. 101° A.M. to 104° P.M. In second week, rose spots appearing on 8th day, nausea on taking food; diazo-reaction questionable. Third week, agglutination test probably positive. Fifth week, 33d day, three epileptiform convulsions at intervals of forty minutes, followed by clonic spasms lasting five minutes, very much more marked on the right side than on the left; pupils equal. Much mental confusion in following two weeks, with marked amnesic aphasia. During eighth week, much better. In ninth week spasms returned, with coma, terminating in death on the 66th day.

Autopsy.—Suppurative basilar meningitis; abscess of left temporal lobe; purulent exudate in lateral ventricles; healing typhoid ulcers in ileum; acute splenic tumor; cloudy swelling of solid viscera; moderate diffuse arteriosclerosis; chronic interstitial nephritis.

Histology.—The organs show the usual typhoid appearances. The wall of the brain abscess shows a capsule, with purulent exudate inside, and regenerative changes outside; and with groups of short blunt bacilli with rounded ends, both inside and outside the capsule.

Bacteriology.—The cerebellar exudate and the cerebral abscess both contain in pure cultures an actively motile bacillus of typhoid-like morphology, which from its growth on differential culture media and from its reactions with typhoid serum, and the action of its specific serum on typhoid and allied bacilli, is evidently *Bacillus typhosus.* The same organism, together with *Bacillus coli communis,* is also present, in the liver and kidney; the lung contains *Staphylococcus pyogenes citreus;* the blood from the heart and the kidney is sterile.

The Diplococcus scarlatinæ: W. J. CLASS, M.D., Chicago, Ill.

The author gives a brief description of a germ which he considers the etiologic factor of scarlet fever. This germ is a polymorphic coccus, usually occurring as a large diplococcus. It stains with any of the ordinary aniline stains, has no capsule and no independent motion. It forms small grayish-white colonies upon a special medium devised by the author, consisting of glycerine agar to which five per cent. of garden earth has been added. It also grows to some extent upon blood serum and in bouillon. It is found in the throat secretions, blood scales and urine of patients suffering from scarlet fever. It is also found in certain cases of angina which are probably scarlatina *sine* eruption. Control experiments have been made showing that the germ is found practically only in cases where contact with scarlet fever patients can be traced. Swine, guinea-pigs and mice are susceptible to the diplococcus. In swine a disease characterized by fever, a red rash and subsequent scaling has been produced by Gradwohl Jaques and the author. Organs from these animals examined histologically by Le Count showed the changes usually found in fatal cases of scarlatina. Experiments were made regarding immunity. These showed that blood from a scarlet fever patient conferred immunity against the *Diplococcus scarlatinæ.* Guinea-pigs were also immunized by means of blood serum from a pig that had been injected with gradually increased doses of the toxin. The author gave the following reasons why he considers this diplococcus to be the causative factor of scarlet fever. (1) Because the germ is invariably present in cases of scarlatina. (2) Because it is a decidedly pathogenic microorganism. (3) Because with it a disease can be reproduced in swine which closely resembles scarlet fever. (4) Because blood serum from a scarlet fever convalescent exerts an inhibitory effect upon the germ, whether in the body or in culture. (5) Because the germ grows in milk without producing any change in the medium. (6) Because the disease produced in mice and swine is contagious. (7) Because the authors' findings have been corroborated by reliable observers so often that errors of observation can be excluded. In closing, the author makes a plea that the germ be given a thorough investigation, as he feels certain that unprejudiced work will show the truth of his statements.

A Contribution to the Physiological Differentiation of Pneumococcus and Strepto-

coccus, and to Methods of Staining Capsules: P. H. Hiss, M.D., College of Physicians and Surgeons, New York.

The author believes that up to the present time it has not been demonstrated that pneumococci and streptococci can at all times be clearly differentiated from each other. Well-marked capsules have been found by various observers to occur on organisms more reasonably classified as streptococci than as pneumococci. On the other hand, capsules may not be demonstrable by the usual methods on pneumococci, especially when these organisms are growing on artificial media. Pneumococcus cultures may also show a predominance of chains, while streptococci may occur in pairs. The usual cultural characters and reactions of these organisms are at the best not diagnostic, and are subject to variations.

Experiments by the author, with cultures of pneumococci and streptococci from many different sources indicate well-marked, constant differences between the metabolic activities of pneumococci and those of streptococci. These differences in metabolism become apparent when the organisms are cultivated in the following media: (1) A medium composed of ox serum, one part; distilled water, two parts; normal sodium hydroxid, 0.1 per cent. (2) A medium composed of ox serum, one part; distilled water, two parts, and inulin, 1 per cent. These serum media are not coagulated by boiling and are sterilized at 100°C. Acid is formed in each of these media by pneumococci when grown at 37°C., and a solid yellowish-white coagulum results. The coagulation is rapid in the inulin medium, slower in the alkaline. Streptococci do not form acid in these media, and no coagulation occurs. These media have, therefore, in all instances, served to differentiate pneumococci from streptococci.

Other mono-, di- and poly-saccharids were tested in media made in the same manner as the inulin medium, but were fermented and the media coagulated, by various members of the streptococcus group, as well as by pneumococci. Hence, these carbohydrates are not of use in differential tests. Special methods were devised for demonstrating capsules on pneumococci and streptococci. Chief among these was to grow the organisms on ascitic serum agar, preferably plus one per cent. glucose. Spread the organisms on the cover-glass by mixing with a drop of serum, or a drop of one of the fluid serum media. Dry in the air and fix by heat. Then stain for a few seconds in a one half saturated aqueous solution of gentian violet. Wash off with a 0.25 per cent. solution of potassium carbonate, mount and study in this solution. This is a good stain for capsules of pneumococci in blood or serum of infected animals. Pneumococcus capsules may also be stained by the following method and be mounted in balsam without injury. A five per cent. or ten per cent. solution of gentian violet or fuchsin (500 sat. alcoholic sol. gentian violet plus 95 c.c. distilled water) is used. This is placed on the dried and fixed cover-glass preparation and gently heated until steam arises. The dye is washed off with a twenty per cent. solution of copper sulphate ($CuSO_4$ cryst.). The preparation is then dried and mounted in balsam. By these methods most streptococci were found to have capsules.

Well-marked examples of encapsulated organisms, such probably as those which have been described by some investigators, and separated by them into new species distinct from *Streptococcus pyogenes,* or with no certainty differentiated from pneumococci, have been examined and have been found to correspond to *Streptococcus pyogenes* in the media mentioned in this paper.

The conclusion from these facts is that such encapsulated organisms should with hesitation be separated from *Streptococcus pyogenes,* unless well-marked cultural differences can be shown to exist.

Branching in Bacteria, with Special Reference to B. diphtheriæ: HIBBERT W. HILL, M.D., Boston Board of Health Bacteriological Laboratory.

The chief problems considered divide themselves into morphological and physiological or developmental problems. The general subject is one of fundamental importance, theoretical and, in its relation to diagnostic work, practical.

The principal hypotheses to be considered as offered to explain branching may be briefly stated: (1) Accidental opposition. (2) Budding from single-celled rods. (3) The turning aside of a medial cell in a chain of closely connected cells, supposed to compose the larger bacterial rods, and the subsequent 'chaining out' of such a cell (Nakanischi's view). (4) The development of a medial melochromatic granule of a new rod. (5) Involuntary changes.

The writer describes the results of the examination of individual bacilli developing in a moist warm chamber under the microscope and concludes: (1) That degenerative (involutionary) changes do at times give rise to distortions distantly simulating branches. (2 and 3) That active branching, by apparent budding resulting in multiplication, does occur in young (five to ten hours old) cultures on agar. (4) That various modifications of the process exist. (5) That such branching may be reversionary or evolutionary in character, but involutionary only in the case noted above (1).

A review of the literature and consideration of nomenclature follow, and a number of drawings are given in the full article.

'Hanging Block' Preparation for Microscopic Observation of Developing Bacteria: HIBBERT W. HILL, M.D., Boston Board of Health Bacteriological Laboratory.

The writer cuts a cube of nutrient agar from a Petri dish full of solidified jelly. The organism to be examined, as an emulsion in water from a solid culture, or as a drop of broth from a liquid culture, is spread upon the upper surface of the agar, as in making an ordinary smear preparation on glass. After drying the cube at 37°C. for ten minutes, a clean cover-slip is applied to the inoculated surface and sealed in place by running a little melted agar round the edges of this surface. The cover-slip is then placed over the opening in the moist chamber, the agar block lowermost, and the microscope focused upon the bacteria. For organisms growing best at 37°C. some form of warm chamber is necessary. The writer describes two such warm stages, devised by himself, and a very simple method of securing a circulation of warm water through them.

A System of Recording Cultures of Bacteria Genealogically for Laboratory Purposes: BURT RANSOM RICKARDS, S.B., Boston Board of Health Bacteriological Laboratory.

The writer applies to the recording of all individual tube cultures of bacteria and the data relating to them a modification of the Dewey Decimal System under card catalogue entries to correspond. Each species is known by some whole number in the hundreds (or thousands if a great many cultures of one species are to be dealt with). Thus:

B. coli=100,	*B. diphtheriæ*=300,
B. typhi=200,	*B. mallei*=400.

Individual specimens of any one species are numbered in the order of their isola-

tion, 1 to 49 (or 1 to 499 if the species are numbered in the thousands). Thus:

B. mallei from one horse=401,
B. mallei from a second horse=402,
B. mallei from a different lesion in the second horse=403.

The first culture isolated pure is given a number in the first place of decimals—thus the pure culture of glanders bacilli from the first horse mentioned would be 401.1.

Subcultures are expressed by the number of the original culture with the figure 1 placed in the next right place of decimals. If further subcultures (sister cultures) are made from the same mother culture, they are differentiated by increasing this last new figure in arithmetical order. The system is very simple, very accurate, very elastic, and, construed under the card catalogues described, saves an enormous amount of work, and ensures a complete record of every tube used.

Variety of the Hog Cholera Bacillus which closely resembles Bacillus typhosus: M. Dorset, Biochemic Laboratory, Washington, D. C. (By title.)

The author described a variety of the hog cholera bacillus which was isolated from a virulent outbreak of hog cholera in Page County, Iowa. This variety corresponds in every way with the hog cholera bacillus as usually seen, except in its action upon glucose, which it ferments without the evolution of gas. The failure to produce a gaseous fermentation of glucose places this variety of the hog cholera bacillus culturally closer to *Bacillus typhosus* than to the hog cholera group of bacteria. A comparison with several cultures of *Bacillus typhosus* has shown that culturally this variety of hog cholera bacillus cannot be distinguished from some of them; but the author concludes that when the source and pathogenic properties of this variety are considered, it should be classed among the hog cholera bacteria.

The Morphology of Bacillus Diphtheriæ: Frederic P. Gorham, Brown University, Providence, R. I.

The experiments described were made for the purpose of proving that the long, granular form of the diphtheriæ bacillus, type C of Westbrook, can be changed into the short, thick, solid-staining, sometimes double-headed bacillus, type D^2 of Westbrook. A pure culture of type C was made from a clinical case of diphtheria. After several platings a culture was obtained that showed only long granular forms, careful search and the use of Neisser's stain failing to demonstrate a single bacillus of any other type. Plates were made from this culture and some thirteen colonies examined. The colony that showed the largest number of shorter forms was selected, and plates made from it. This process of selection and plating was continued for some fifteen generations.

The colony of the fourth generation was composed of forms distinctly smaller than those of the original culture; all were still granular, however, and stained by Neisser's method. In the fifth generation, some of the bacilli failed to show granules by Neisser's method, were barred, and of the type C of Westbrook's. In the eighth generation many of the bacilli failed to react to Neisser's stain, and a large number of barred and solid staining forms were present. Some of these approached the double-headed type D^2. On continuing the selection until the fifteenth generation all the granular forms were eliminated and the colony became a pure culture of the type D^2; the majority of forms were of the double-headed variety.

The experiment is being continued by testing the various types for virulence. From the results already obtained it ap-

pears that the different forms of the *Bacillus diphtheriæ* can be produced by the variations of a single type.

An objection to the validity of these results, of course, can be made by the claim that pure cultures of the various types were not used. The objection seems to me not well taken, on account of the large number of platings and the fact that several generations were passed before any change appeared. These experiments are directly in line with the results already obtained by the author in the study of the changes of form observed in the diphtheria bacillus in the noses and throats of persons immune or becoming so.

A Note on Branched Forms of Tubercle Bacilli Found in Cultures: M. DORSET, Biochemic Laboratory, Washington, D. C.

The author describes branched tubercle bacilli found in a six weeks' old bouillon culture of human tuberculosis which had become contaminated with a streptothrix. The branches were always Y-shaped and, from various stages in the branching found in cover preparations, the author concludes that the branching has probably taken place in the following manner: The end of a rod first enlarges and then probably separates into two small knobs, which forms have been seen in the cover preparations. These separate knobs grow out and constitute the branches.

An Undescribed Pathogenic Diplococcus: H. GIDEON WELLS, Chicago University.

This was obtained first in cultures from a subcutaneous abscess on the thigh of a woman, aged twenty-two, with the following history: When sixteen years of age her hand was badly cut by a piece of window glass. The wound became infected and it was nearly a year before it entirely healed. During this time she developed a number of subcutaneous abscesses on other parts of the body, which healed slowly. During the six years that have followed she has had, at varying intervals, seldom more than three months, recurrences of the subcutaneous abscesses, which have appeared at one time and another, over almost the entire body. They appear independent of any injury or other known exciting cause, and produce only slight constitutional symptoms. The patient is in fair health otherwise, and is able to work except when an abscess is developing. Physical examination gives no information. Blood count shows 3,862,000 red and 7,100 white corpuscles two hours after dinner. Cultures from a vein gave the *Diplococcus* in pure culture at a time when the patient was free from abscesses. Inoculated into animals, the *Diplococcus* sometimes fails to cause any change. Sometimes it produces a slow emaciation which does not terminate fatally, but more often it causes local abscesses. The most characteristic fact is that it can be obtained from the heart's blood of these animals long after subcutaneous or intraperitoneal inoculation, even when there have been no local lesions, and when the animal seems well. It often produces abscesses at the sites of injuries several weeks or months after inoculation; in one case, after four months.

Morphologically, it resembles the *Gonococcus* in fresh cultures, sometimes later, becoming more a double sphere. It seldom forms chains or tetrades, does not destain by Gram's method, possesses a capsule that is difficult of demonstration in culture, grows well on all ordinary media, liquefies gelatin after three or four days at 24°C., with production of a funnel-shaped excavation filled with fluid. It produces no gas nor indol; slowly acidifies milk; produces no pigment; its growth is slimy and rather tenacious both in solid and liquid media.

The Distribution of B. coli communis in Natural Waters: C. E. A. Winslow, Massachusetts Institute of Technology, Boston, Mass.

The work of certain observers has shown that organisms apparently identical with *B. coli communis* are found widely distributed in nature. The problem for the bacteriologists, and especially for the sanitarian, becomes, then, a quantitative one. Even if this organism does occur in various places in the outside world, may it not still be true that it thrives only, or at least most abundantly, in the intestine of the higher vertebrates, and that an overwhelming proportion of the individual representatives of the species occurs in that habitat? May it not be true then that while the *Bacillus coli* is found at times in unpolluted waters, its presence, constantly, or in numbers, is still characteristic of sewage pollution? As a preliminary contribution to this question a number of presumably unpolluted waters in the neighborhood of Boston have been examined for the *Bacillus coli.* In each case one centimeter of the water was incubated in dextrose broth and one hundred cubic centimeters were incubated with the addition of phenol-glucose broth. From the one hundred cubic centimeter bottles, after incubation, dextrose broth was inoculated, and from all dextrose tubes showing gas, litmus-lactose-agar plates were made. Three characteristic colonies were fished from each reddened litmus-lactose-agar plate, and subcultures inoculated. Only those which gave the following reactions were considered as *B. coli:* The fermentation of dextrose broth with the production of gas in twenty-four hours; the fermentation of lactose in the litmus-lactose-agar plate, with distinct reddening in twenty-four hours; the coagulation of milk in twenty-four hours; the production of nitrite from nitrates in twenty-four hours; the production of indol in peptone solution in three days; the formation of an abundant growth covering nearly the whole surface of the agar streak in twenty-four hours, later becoming whitish and cheesy, but not stringy to the needle; the formation of round or oval white colonies in the gelatin shake culture, often with gas bubbles, with no liquefaction of the gelatin in seven days. The use of one hundred cubic centimeters appears in these experiments to have increased the proportion of dextrose and lactose fermenting organisms, but not of colon bacilli. The investigation included the study of seventy-nine samples of water. The results lead to the conclusion that the colon bacillus is so rare in normal unpolluted waters as to be found infrequently when single centimeters of the water are examined. The presence of this organism, constantly, or in a majority of cases, in one cubic centimeter, may still be assumed to be due to the entrance of some polluting substance.

Preliminary Observations on B. coli communis from Certain Species of Animals: Veranus A. Moore and Floyd R. Wright, Cornell University, Ithaca, N. Y.

A study has been made of *B. coli communis* found in a single place in each of the large and small intestines of nine horses, eleven cattle, eight sheep, four swine, eight dogs and six chickens. This organism was not found in the intestines of six frogs, two young and one old rabbit. It had previously been found in several rabbits. These animals were all supposedly healthy and, excepting the frogs and rabbits, were for the greater part killed for dissection or food. Cultures in bouillon from six colonies from the gelatin plate cultures from each of the large and small intestines were made; these were replated, and from the colonies which developed on them subcultures were made and studied on gelatin, agar and potato; in milk,

bouillon, and in bouillon containing one per cent. dextrose, lactose and saccharose. The degree of motility and the indol reaction were also considered.

The purpose of these examinations was to find the extent to which varieties of this bacillus exist normally in the intestines of both different individuals of the same species and of different species of animals. This was to determine if the many varieties of the colon bacillus which have been described from polluted water, soil and from lesions of various kinds in man and animals, have their natural existence in the generally supposed normal habitat of this species of bacteria. The results showed no pronounced variation in the morphology or the cultural characters of these bacilli from different sources on gelatin, agar, potato and bouillon. The action on the sugars, milk and the indol production differed somewhat. The two varieties, A and B, described by Smith in 1896 were found. The A variety, *i. e.*, those that ferment, with gas production, dextrose, lactose and saccharose, and the B variety, *i. e.*, those that do not ferment saccharose, were found in about equal numbers in each species of animals. Other varieties did not appear, although there were slight variations in the quantity of gas, the gas formula, and the time required for the milk to coagulate. The cultures from different colonies from the same plates did not show any appreciable difference except in one instance, from a dog. The plate cultures made from one dog did not develop colonies resembling those of *B. coli communis*. The cultures from dogs were more virulent for guinea-pigs than those from the other species of animals. The action on the sugars was considered of the most differential importance.

Color Standards for Recording the Results of the Nitrite and Indol Tests: C. E. A. WINSLOW, Massachusetts Institute of Technology, Boston, Mass.

In studying the effect of certain external conditions on the reactions of the *Bacillus coli communis*, need was felt of some definite standard by which to measure somewhat quantitatively the capacity for nitrite and indol production. It is true that even when the conditions of the experiment—the composition of the medium, the amount and character of the culture used for inoculation, and the time allowed for the development of the reaction—are rigidly controlled, striking variations sometimes appear. The laws of such variations can, however, only be properly studied when their sequence is made manifest by definite comparable standards.

The use of a color standard for measuring the reduction of nitrate and the formation of indol obviously suggests itself as simpler and more practical than any other method. Up to the point at which a precipitate forms in the nitrite reaction, the depth of color in both cases may be considered as roughly proportional to the amount of the end product formed by the bacteria in a given time. The problem for the bacteriologist is then to select from the numerous schemes of color values, prepared for artistic and educational purposes, that one best suited for the matching of the reaction in question.

The most rational system of color standards is that prepared by Milton Bradley, of Springfield, Mass., based on pure spectral colors of known wave-length. It is issued in the form of a small booklet, and by cutting out and pasting to a card the colors between red and yellow, orange and their tints, a chart is obtained on which the color of the indol reaction produced by *B. coli communis* can be readily matched. The hue is read by holding the tube parallel to a white surface and looking through it at right angles, while the matching color on the

card is isolated by a small card with a window cut in it. The tube and card are viewed in strong diffuse daylight. The Milton Bradley color scheme has not, however, proved satisfactory for measuring the reduction of nitrates. A large majority of the tubes tested lay somewhere between the reds and the violet reds of the scale, and could not be well matched with either. Some other standards were therefore sought and more suitable ones found in the book of standard colors published by Louis Prang, Boston, Mass. This system has no definite scientific basis. The hues are less pure and the tints less bright and clear than in the Bradley system; the gradations, however, are more numerous. Of the seven plates in the Prang book the last five, including the darker shades, are not needed. On the 'pure color' plate and the 'first shade' plate the colors produced by both the indol and the nitrate test can be quickly and easily matched. As far as this standard has been used it has been found to be a satisfactory system of record and an important aid in forming definite ideas as to the behavior of microorganisms under various conditions.

Observations upon the Morphological Variation of Certain Pathogenic Bacteria: A. P. Ohlmacher, M.D., Northwestern University Medical School, Chicago, Ill.

Three observations are here recorded. The first was upon experimentally-induced morphological variation in *B. diphtheriæ*. Here a race, presenting the long, granular or barred type, was transformed to the short, solid type by a forty-eight-hour sojourn in the subcutaneous tissue of a white rat. Two originally short, solid organisms were converted into long, granular ones by a single passage through the organs of a guinea-pig. In the second observation a race of *Streptococcus pyogenes* from a case of follicular tonsillitis was observed to assume the form of a large polymorphous bacillus each of the seven times it was transferred to Loeffler's medium, each time resuming the morphology of the ordinary *S. pyogenes longus* when grown in bouillon. A race of *B. coli communis* recovered from a case of gangrenous cholecystitis and cholangitis is concerned in the third observation, in which the organism showed a remarkable polymorphism in the original smears and early generations of cultures, taking on a diversity of forms from long, quite coarse filaments to excessively minute coccoid or diplococcoid organisms.

Special Laboratory Apparatus: Wm. R. Copeland, Spring Garden Water Works, Philadelphia, Pa.

The bacteriological laboratory at the Testing Station of the Bureau for Improvement, Extension and Filtration of the Water Supply in Philadelphia, has been equipped with the special object of making examinations of water. Therefore a thermostat was designed and built under special directions. The interior chamber is divided by perforated partitions into four sections. The glass doors, which hang directly in front of these sections, are divided in such a way that the door in front of one section may be opened without exposing the interior of the other sections to the temperature of the laboratory. Between the walls of the interior chamber and the surrounding jacket, strips of copper are soldered, so that water pumped into the open space between the walls must circulate round all four sides of the interior, before it can escape through the drain.

The apparatus employed at the Testing Station for filling test-tubes consists of a copper funnel screwed on to a brass pipe, which, in turn, is screwed on to the top of a brass cylinder. Inside of the cylinder is a brass plug containing two holes, one holding ten, and the other five, cubic centi-

meters. The plug fits snugly inside of the cylinder, but may be turned from right to left easily by a lever attached to a post on top of the plug. This post passes through a hole in the cap. A delivery tube is screwed on to the bottom of the cylinder, and is attached at a point 90° from the opening in the top cap to the funnel. Directly over the delivery tube a little hole is drilled through the cap to serve as an air vent.

When one of the chambers in the plug has been filled with the medium, the lever is reversed and, as the chamber passes over the delivery tube, the medium is discharged into a test-tube below.

An Unusual Bacterial Grouping: MARY HEFFERAN, University of Chicago, Chicago, Ill.

An organism presenting peculiar morphological characteristics, obtained from Kral's Laboratory Collection, Prague, was sent under the name of *B. rosaceus metalloides*. This organism produces an orange-red pigment, but differs from the original description of the above-named form in three important cultural features, viz., lack of metallic luster, for which *B. rosaceus metalloides* is peculiarly known, non-liquefaction of gelatin to any active degree, and possession of the power of motion. A hanging drop preparation from a bouillon culture of seventy-two hours, room temperature, shows the characteristic grouping to best advantage. Short small bacilli, about the size of *B. acidi lactici*, are seen grouped together in aster-like clusters varying in composition from but three or four bacilli up to fairly compact spherical burrs of rodlets radiating from a center. The number of these asters increases in a culture for several days, especially at the surface, where they are loosely packed to form a reddish surface scum. There seems to be nothing like a capsule or a gelatinous zoogloea massing. Below the surface a greater number of free motile bacilli are present. In old cultures the asters are irregular and fewer in number, free bacilli predominating. On solid media the aster formation has been observed only on agar cultures. The bacilli are longer and more slender than in bouillon, and, when stained, show the *Volvox*-like grouping very beautifully. On potato the bacilli are short and thick, and may show well-defined capsules enclosing two rods.

Observations on the important question as to whether the asters are formed by cell division as a process of growth, or by a method akin to agglutination, are as yet incomplete. The regularity of the formation, the occurrence on solid as well as in liquid media, and the final disappearance of the asters, point, however, to the conclusion that this phenomenon is a growth phase in the life-history of the organism.

An Improved Method of making Collodion Sacks: C. S. GORSLINE, M.D., University of Michigan, Ann Arbor, Mich.

Apparatus.—A long test-tube or other tube, closed at one end except for a perforation 2–4 mm. in diameter, and having a caliber corresponding to the diameter of the desired sack; a wide-mouthed 6–8 oz. bottle one-third full of colorless collodion of U. S. P. strength, and a few ounces of distilled water.

Manipulation.—Touch the perforated end of the tube to the surface of the collodion, thereby obtaining a film of collodion over the opening, but none inside. Allow this to dry a few moments. Incline the bottle containing the collodion as much as possible without spilling, and insert the tube, rotating slowly, allowing only the lower one-fourth to be immersed in the collodion. Withdraw from time to time to allow partial drying to take place, repeat-

ing the operation until the desired thickness is reached. As soon as the collodion has set, the tube may be repeatedly immersed in water at about 25°C. to hasten the drying of the sack. When this is accomplished pour distilled water into the open end of the tube, and applying the mouth, force the water, which carries the collodion ahead of it, through the perforation in the tube. The water now creeps in between the sack and the tube and this process is aided and made to progress evenly on all sides by slightly twisting back and forth on the free end of the sack. When the water has traversed the entire length of the sack, the latter slips off easily into the hand. The water, by its pressure, not only releases the sack, but tests it for weak places or perforations at the same time. Tubes may be made from one sixteenth inch to two inches in diameter and twenty inches long. 200 c.c. of saturated magnesium sulphate were dialyzed out through such a sack against running water in four hours and twenty minutes. Parchment requires seven to eight days to accomplish the same.

Neutral Red in the Examination of Water: Ernest E. Irons, University of Chicago.

In 1898 Rothberger found that *B. coli communis* will reduce neutral red in a culture medium, changing the color from red to a canary yellow, with an accompanying green fluorescence. Schleffler tested a number of races of *B. coli* and found that all gave the neutral red reaction. In 1901 Savage employed neutral red for the detection of *B. coli communis* in water. He concluded that a positive reaction, obtained with neutral red, while not certainly diagnostic of *B. coli*, yet in the vast majority of cases, points to the presence of that organism, and that in the case of the fifty waters examined, the margin of error in assuming that *B. coli* was present where a positive neutral red reaction was obtained, was less than five per cent.

The object of the present experiments was to determine further the value of neutral red in the routine examination of water. Following the suggestion of Savage, ordinary bouillon was used, to which was added one-half per cent. of dextrose and one per cent. of a one-half per cent. aqueous solution of neutral red. All cultures were kept at 37°C. Determinations were made by the dextrose fermentation tube and neutral red methods in exact parallel. Samples of forty-five waters were employed with a number of dilutions of each, such that in the case of each water *B. coli* was almost always found in the lowest, and rarely in the highest dilution. In this series 285 determinations were made by either method, with thirty-five per cent. positive results for the fermentation tubes and forty-seven per cent. positive with neutral red. Of the neutral red tubes showing positive results when the corresponding fermentation tubes were negative, 31 were examined for *B. coli*. From five of the 31 typical *B. coli* were isolated, and from 25 organisms were isolated which differed more or less from *B. coli*, but which gave the neutral red reaction. Of these 25, 18 gave no gas in dextrose bouillon. In all, 122 cultures were examined. Of 17 conforming culturally to *B. coli*, 15 gave a positive and two only a very slight reaction with neutral red. Of eleven gas-producing organisms, differing slightly from *B. coli*, four gave positive and seven negative reactions. Four organisms conforming culturally to *B. cloacæ* gave complete reactions. Of 65 non-gas-producers three gave decided, and 24 partial, reactions with neutral red.

The results show that the neutral red reaction is produced, under the conditions of the test, by a number of water bacteria,

which no classification, however liberal, would place in the colon group.

A Graphical Tabulation of the Morphological, Cultural and Biochemical Characters of Certain Bacteria, together with References to Authorities, Synonyms, Literature, Etc.: ARTHUR I. KENDALL, S.B., Massachusetts Institute of Technology, Boston, Mass.

Great difficulty is experienced by bacteriologists in establishing the identity of a given bacterium whose morphological, cultural and biochemical characters have been worked out. This is due, as has already been shown, to the following facts: (*a*) Inaccessibility of the literature, (*b*) incompleteness of descriptions and indefiniteness of terms used, and (*c*) lack of uniformity, in both the choice and composition of media. The object of this paper is to collect in one set of tables the descriptions of as many bacteria as possible, tabulated after each individual characteristic, has been verified by comparison with the leading authorities, using (*a*) terms that admit of one and only one interpretation, and (*b*) terms that will exclude as far as possible the *personal factor*. The graphical method of tabulation first proposed by Fischer ('Vorlesungen über Bakterien'), and first used as a means of classification by Fuller and Johnson (*Journal of the American Health Association,* Vol. XXV., p. 580 et seq.), has been adopted because the definiteness of characteristics attained with this method of representation cannot be equalled by any other known method. Tables were shown, including those micrococci, which do not liquefy gelatin and do not produce pigment—these being the first of a set of tables and references in which the author hopes to include the more common forms of bacteria.

As a result, even a cursory glance at the tables show the great similarity between certain bacteria that are supposed to be different species. For example, nine species of micrococci were referred to table 2. These bacteria were isolated and described by the same authority, were obtained from different varieties of cheese, were supposed to be separate species, and yet the characteristics shown by this method are identical, and even the written descriptions point strongly to the fact that these 'species' are, at best, only varieties of the same form.

Some Experiments with Synthesized Media: M. X. SULLIVAN, Brown University, Providence, R. I.

Pasteur, Cohn and others recognized that some bacteria can secure their carbon, hydrogen, oxygen and nitrogen from simple compounds. This is to be expected from the close relationship of the bacteria to other plants. Recently the question of simple synthesized media has received some attention from Kuntz, Jordan and de Schweinitz. Since Pasteur, however, little use has been made of media other than those made of meat infusion and peptone, with agar or gelatin as a base. Meat infusion and commercial peptone vary so widely in their chemical composition and in their nutrient value, that media composed of these substances are practically of no use in the study of pigment or antitoxin production or in the study of bacterial metabolism. Analysis of standard bouillon shows such small amounts of albumoses and peptones that it appears as if we might neglect the meat infusion entirely, or at least replace the peptone by a non-nitrogenous, non-albumenous body.

On medium (A) consisting of water 100 grams, Witte's peptone 5 gms., NaCl 3 gms., agar 1 gm., 17 different kinds of bacteria, including *Microspora comma, B. anthracis, B. typhosus* and *B. coli,* grew more slowly than on the standard media.

Chromogenic bacteria, as *B. pyocyaneus* and *M. pyogenes citreus*, however, failed to produce pigment. On medium (B), made of water 100 gms., peptone 6 gms., NaCl 2 gms., $MgSO_4$ 0.3 gm., K_2HPO 0.5 gm., agar 2 gms., 15 varieties grew quickly with the production of pigment and, in one case, of phosphorescence. Then the peptone was replaced by a non-albumenous compound, such as ammonium salts or asparagin. On medium (C) containing water 100 gms., glycerin 50 gms., $(NH_4)PO_4$ 10 gms., Na_2HPO_4 1 gm., $MgSO_4$ 0.2 gm., agar 1 gm., 19 varieties grew rather slowly. On medium (D) consisting of water 100 gms., asparagin 1 gm., NaCl 0.5 gm., $MgSO_4$ 0.3 gm., agar 1 gm., 9 varieties grew with no formation of pigment. On medium (E) consisting of water 100 gms., asparagin 1 gm., Na_2PO_4 0.1 gm., NaCl 0.2 gm., agar 1 gm., 20 different kinds of bacteria, mostly pathogenic, grew as quickly as on the standard media.

Further experiments are now being carried on and, in view of the fact that so many bacteria can grow on this non-albumenous medium, it is probable that some combination of simple chemicals can be found that will replace the ordinary meat infusion. A medium consisting of such compounds, qualitatively and quantitatively known, would be of great value in the study of bacterial metabolism.

A Tank for the Growth of Germs in Large Numbers: Victor C. Vaughan, Ann Arbor, Mich.

Professor Vaughan, of the University of Michigan, described an apparatus, devised by himself, for the purpose of obtaining bacterial cells in large quantity. A copper tank ten feet long, two feet wide, and four inches deep, with a trough around the edge one inch deep, is covered by a top of the same material. This tank is supported by an iron frame with legs ten inches high, and the whole is placed on a table covered with galvanized iron. A similar tank, two inches shorter and two inches narrower, also provided with a trough around the edge and a cover, sets in the larger one, and is separated two inches from the bottom of the larger one by iron bars extending from side to side. The bottom of the outer trough is filled with water, and the seal trough of the outer tank is also filled with water, while the seal trough of the inner tank is filled with glycerin. Both lids are raised and lowered by wire ropes passing through pulleys in the ceiling. It is necessary that the tanks should be set perfectly level. Twenty liters of two per cent. agar are placed in the inner tank; both lids are lowered, and with large burners underneath the apparatus becomes a sterilizer. After repeated sterilizations, the upper lid of the outer tank is raised and the agar inoculated by pouring through tubular openings, in the top of the inner tank, a liter of a beef-tea culture of the germ. These openings are then sealed with wax and the outer lid lowered. With gentle heat underneath the apparatus becomes an incubator.

After the germ has grown for fourteen days or longer in this tank, the germ substance can, by the addition of a little water, be scraped from the agar, and from each tank there may be obtained forty or more grams of dried, pulverized germ substance.

With the colon bacillus the following facts have been learned concerning its toxin: (1) The toxin is contained within the cell from which it does not, at least under ordinary conditions, diffuse into the culture medium. (2) The toxin is not extracted from the cell by alcohol or ether. (3) Very diluted alkalis do not extract the toxin from the cell. (4) The germs may be heated in sealed tubes with water to 184° for thirty minutes, without loss of